

Miniaturized Time-of-Flight Mass Spectrometers with a Broad Mass Range

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(originally: Mass-Correlated Acceleration for the Analysis of Mixtures)

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JOHNS HOPKINS
MEDICINE

The analytical problem:

- polymer distributions cover a very broad mass range
- multiplex advantage: the TOF mass spectrometer has the ability to record the entire mass range from each ionization event without scanning
- focusing, or mass resolution, in a TOF is effected by the initial spatial and kinetic energy distributions of the ions
- space focusing can be achieved across the entire mass range with a single geometry and set of voltages
- kinetic energy focusing is achieved by pulsed extraction but is mass-dependent

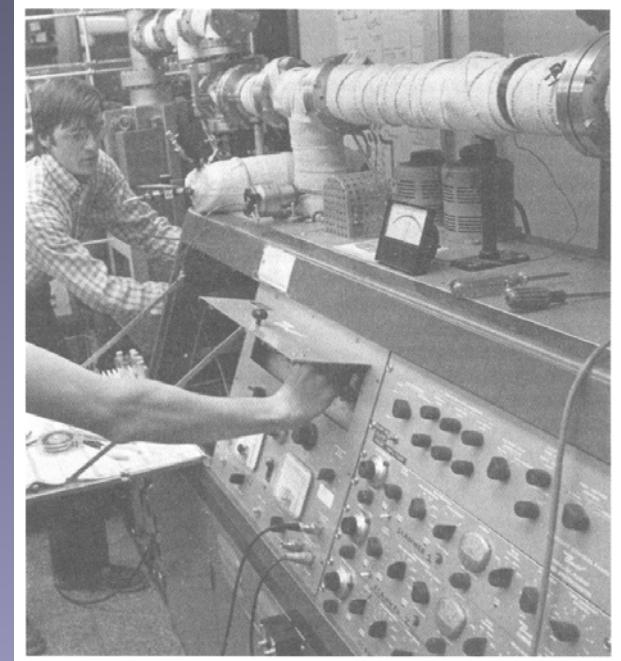
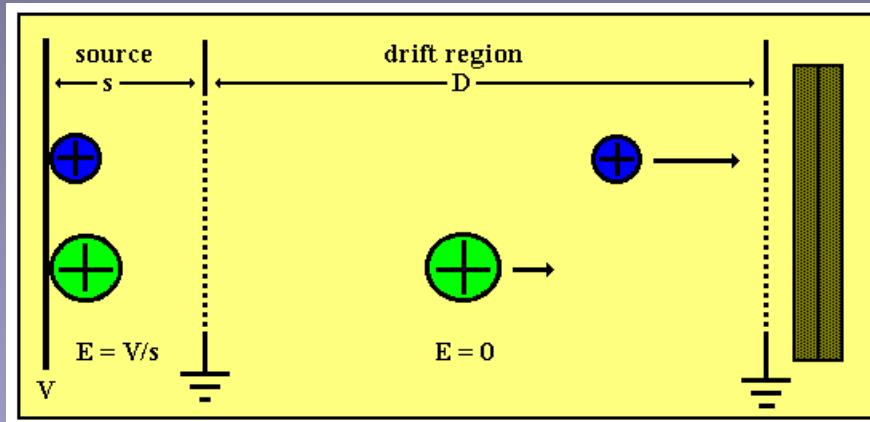
The challenge:

- we would like to achieve broad mass range focusing on a miniaturized instrument (2" to 4" mass analyzer)
- maintaining mass resolution on a small instrument at any mass is itself a challenge

The solutions:

- fixed delay pulsed extraction on a 3" linear mass analyzer with approximately 2nd order kinetic energy focusing at m/z 5,000
- dynamic focusing using mass-correlated acceleration to compensate for different focusing parameters at different masses
- non-linear field mass spectrometers
- the examples used in this work will generally be mixtures of peptides and proteins

The time-of-flight mass spectrometer is the simplest of all mass analyzers:



Ions formed in the ion source (s) appear at the detector with flight times through the drift region (D) proportional to the square root of their mass/charge:

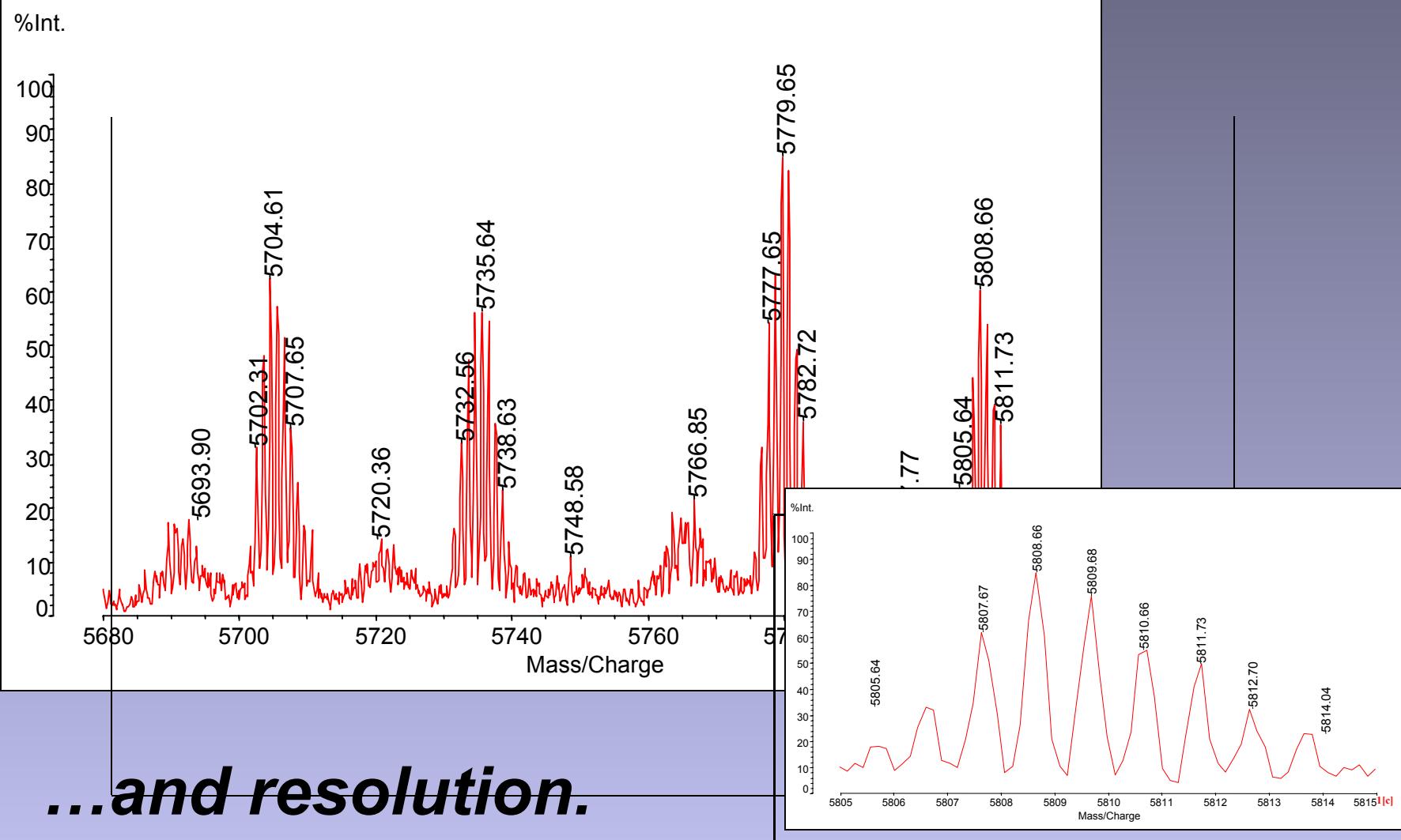
$$t = \left(\frac{m}{2eV} \right)^{1/2} D$$

What are the issues for miniaturization?

*Generally,
time-of-flight
mass
spectrometers
have become
relatively large
instruments ...*



...with very high mass range...



The effects of initial energy and location ...

$$t = \frac{(2m)^{1/2}}{eE} \left[(U_0 + eEs)^{1/2} \mp U_0^{1/2} \right] + \frac{(2m)^{1/2} D}{2(U_0 + eEs)^{1/2}} + t_0$$

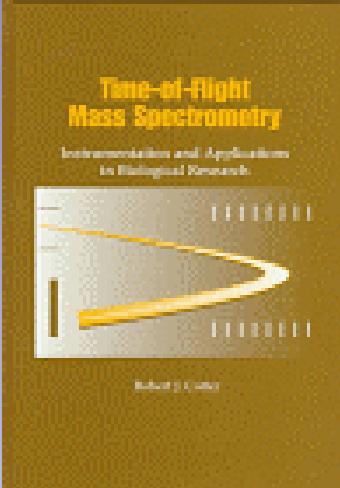
time in ion source **time in flight tube**

initial kinetic energy distribution

turn-around time

distribution of initial position in the source

distribution in time of ion formation



Strategy for a miniaturized TOF MS using a fixed delay pulsed extraction

Development of the 3-inch linear TOF mass spectrometer



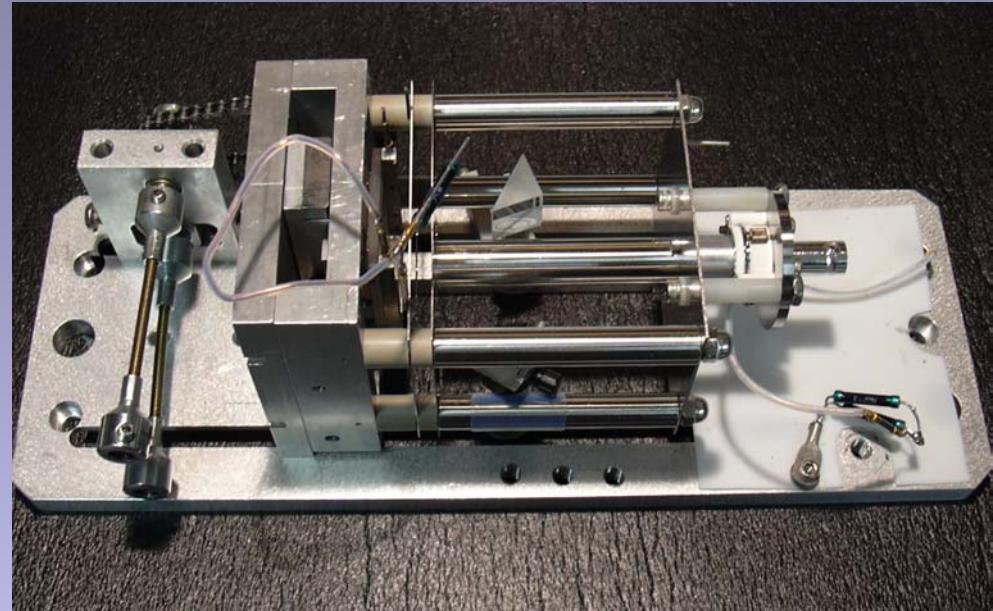
maintain highest possible resolution



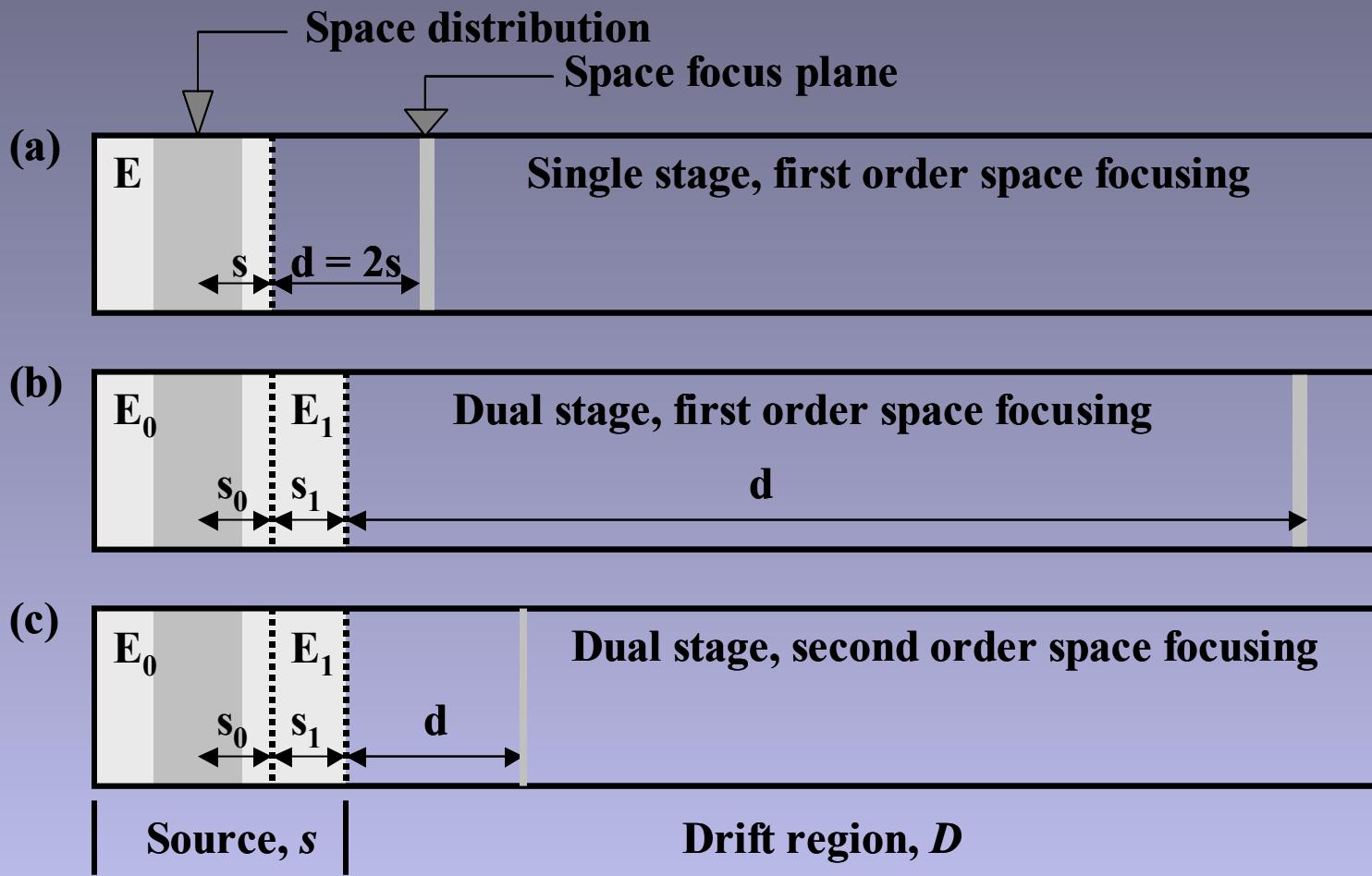
maintain high mass range



design strategy



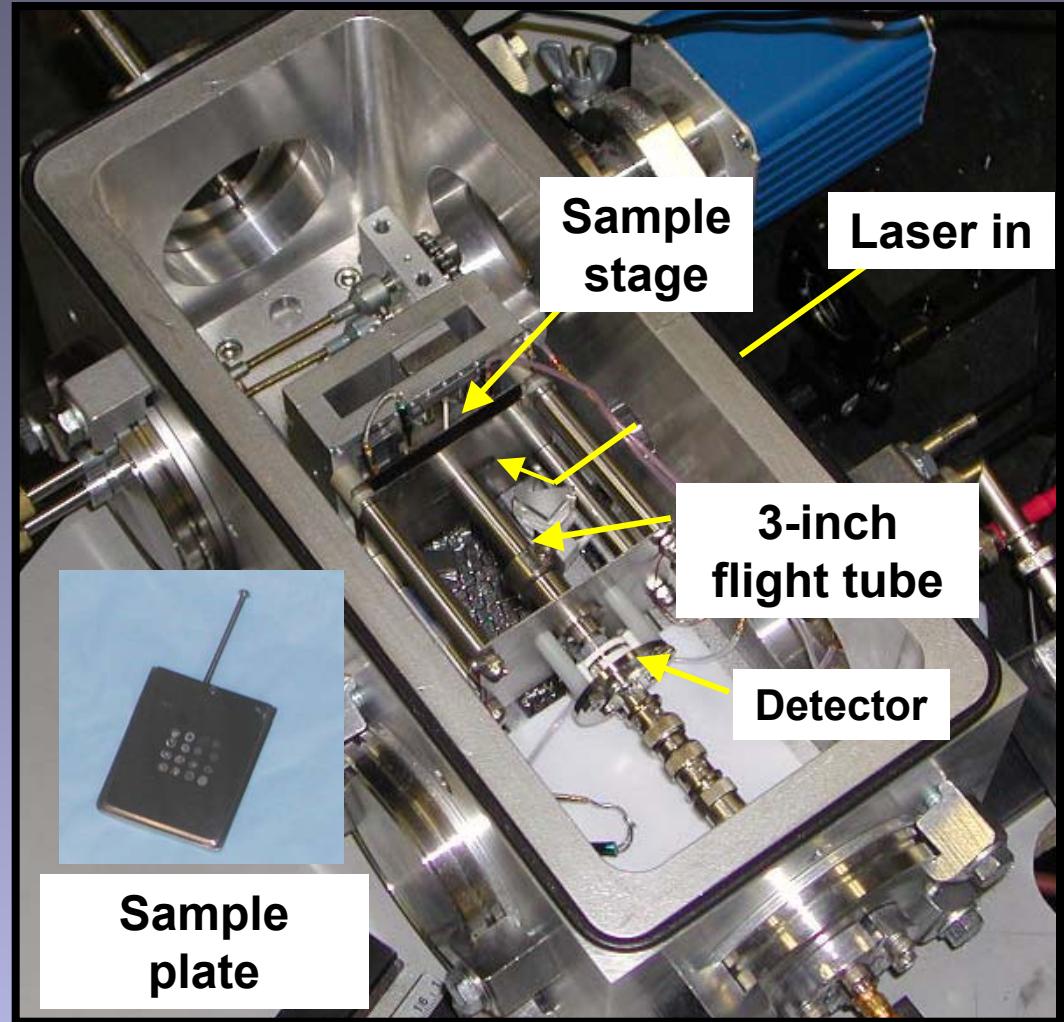
2nd order space focusing occurs at a short distance



Miniaturization Strategy

**Delayed extraction
results in both spatial
and energy
distributions**

**But, the strategy is
similar: do not shrink
the ion source and
provide high order
focusing to a detector
located at a relatively
short distance.**

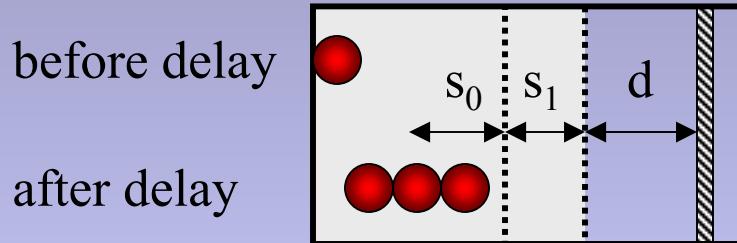


A similar strategy is used for pulsed or delayed extraction focusing:

During the delay, ions with different energies array themselves in different locations, though they still retain their energy distribution.

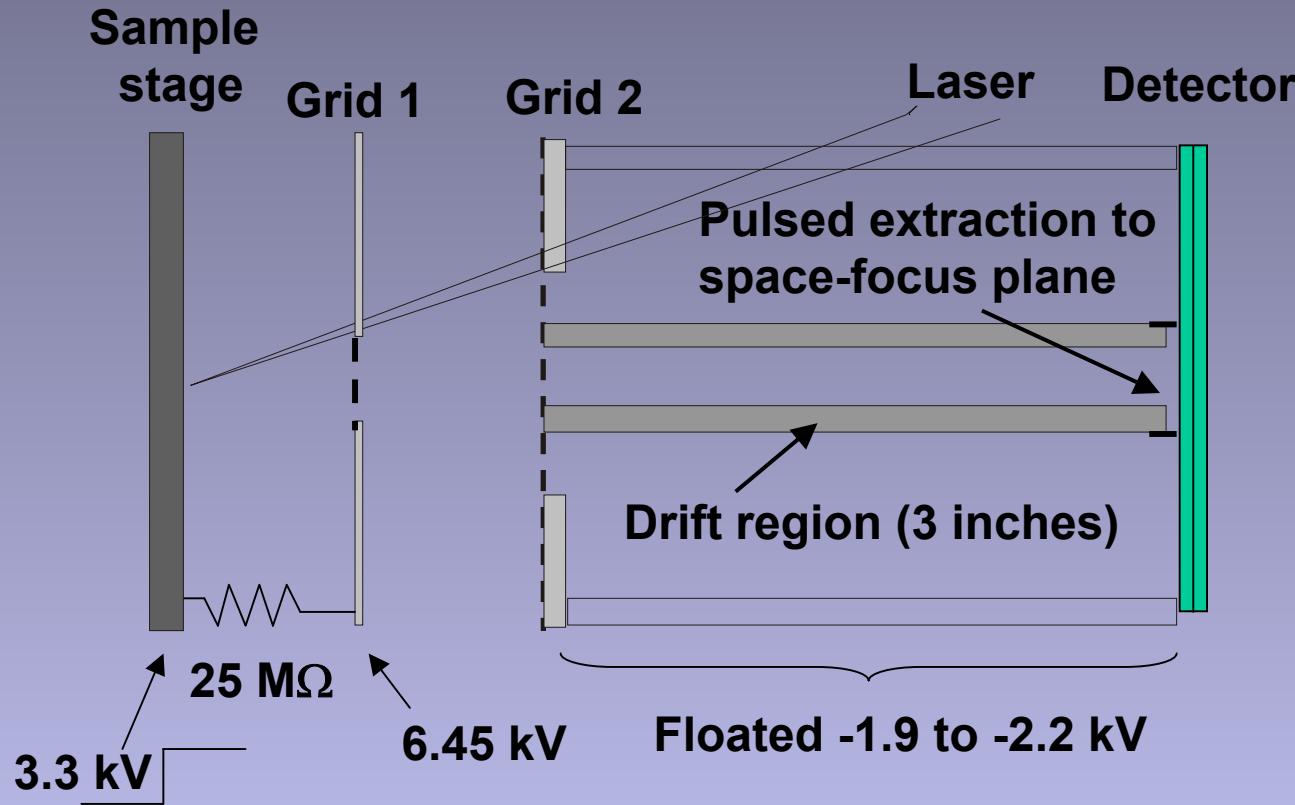


- High order focusing can be used to provide a short focal distance.

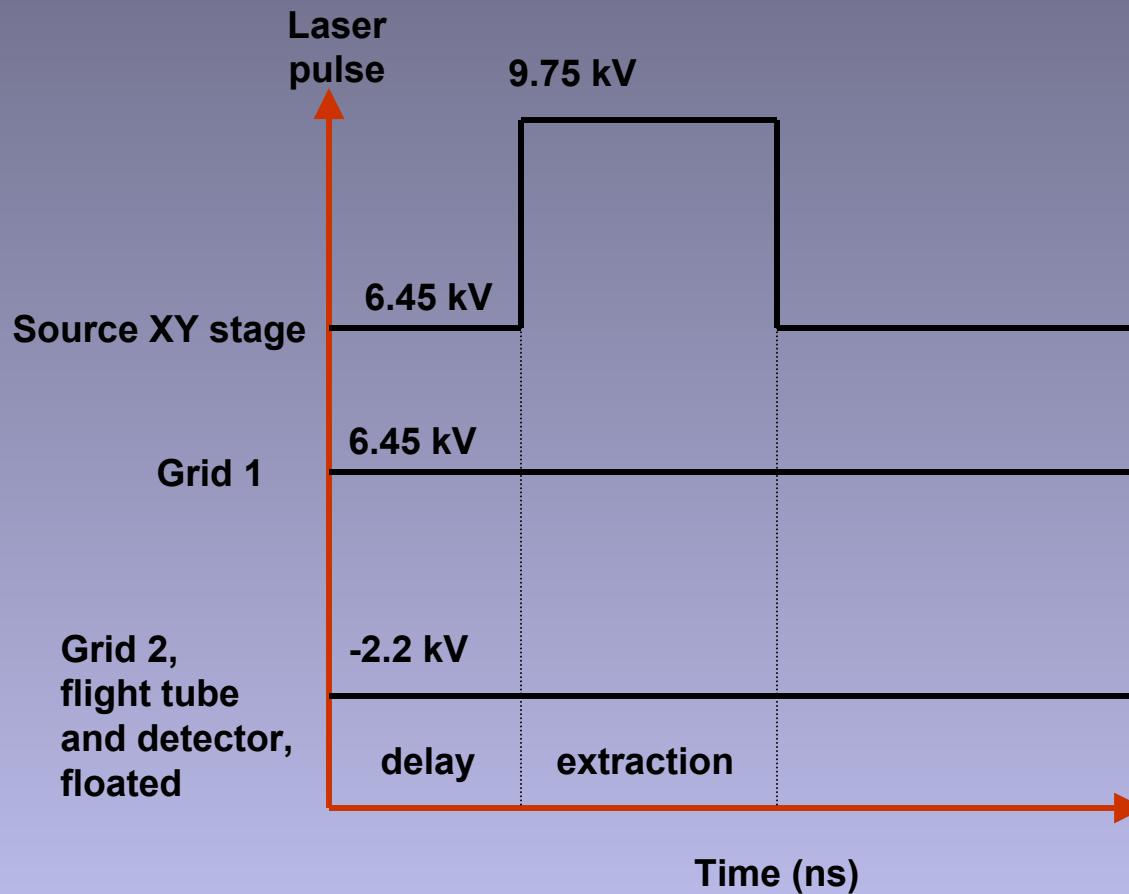


Miniaturized TOF Mass Spectrometer

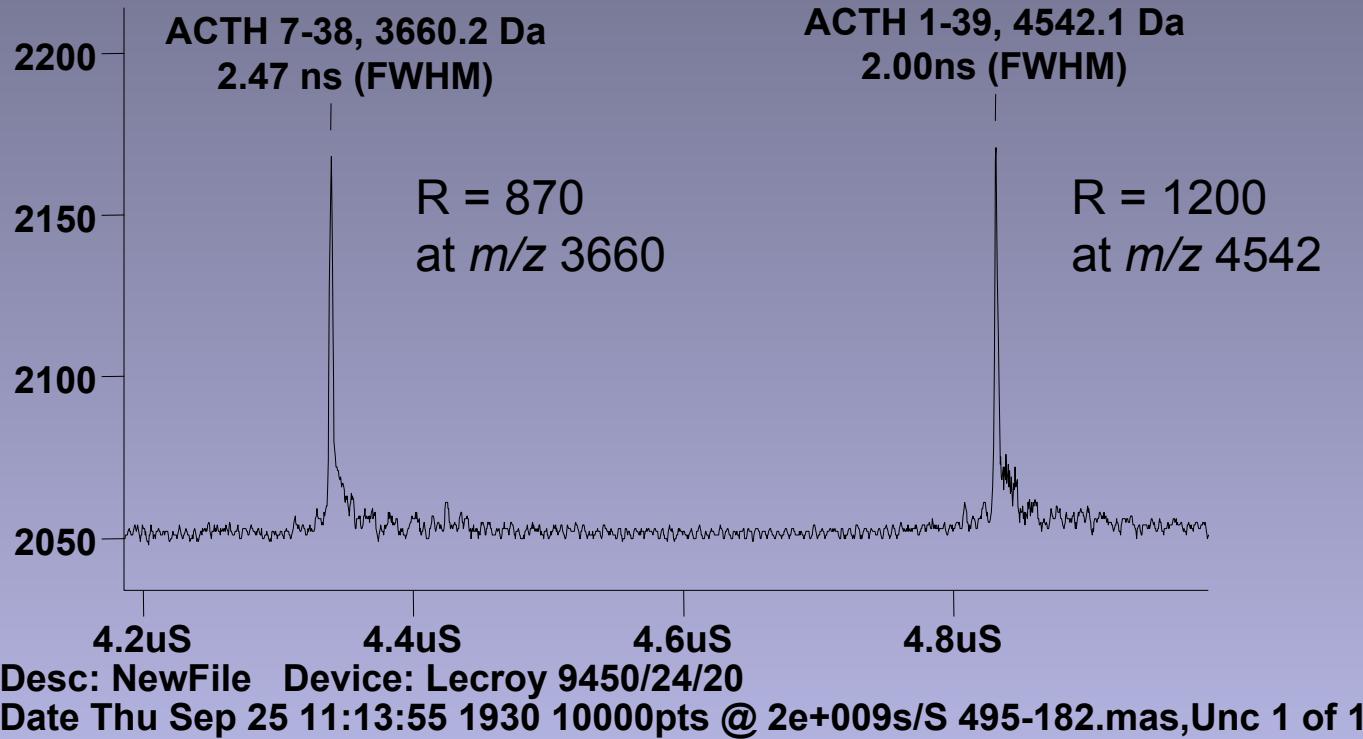
And, float the flight tube to prevent any post-acceleration



Pulsed extraction on the Miniaturized TOF Mass Spectrometer

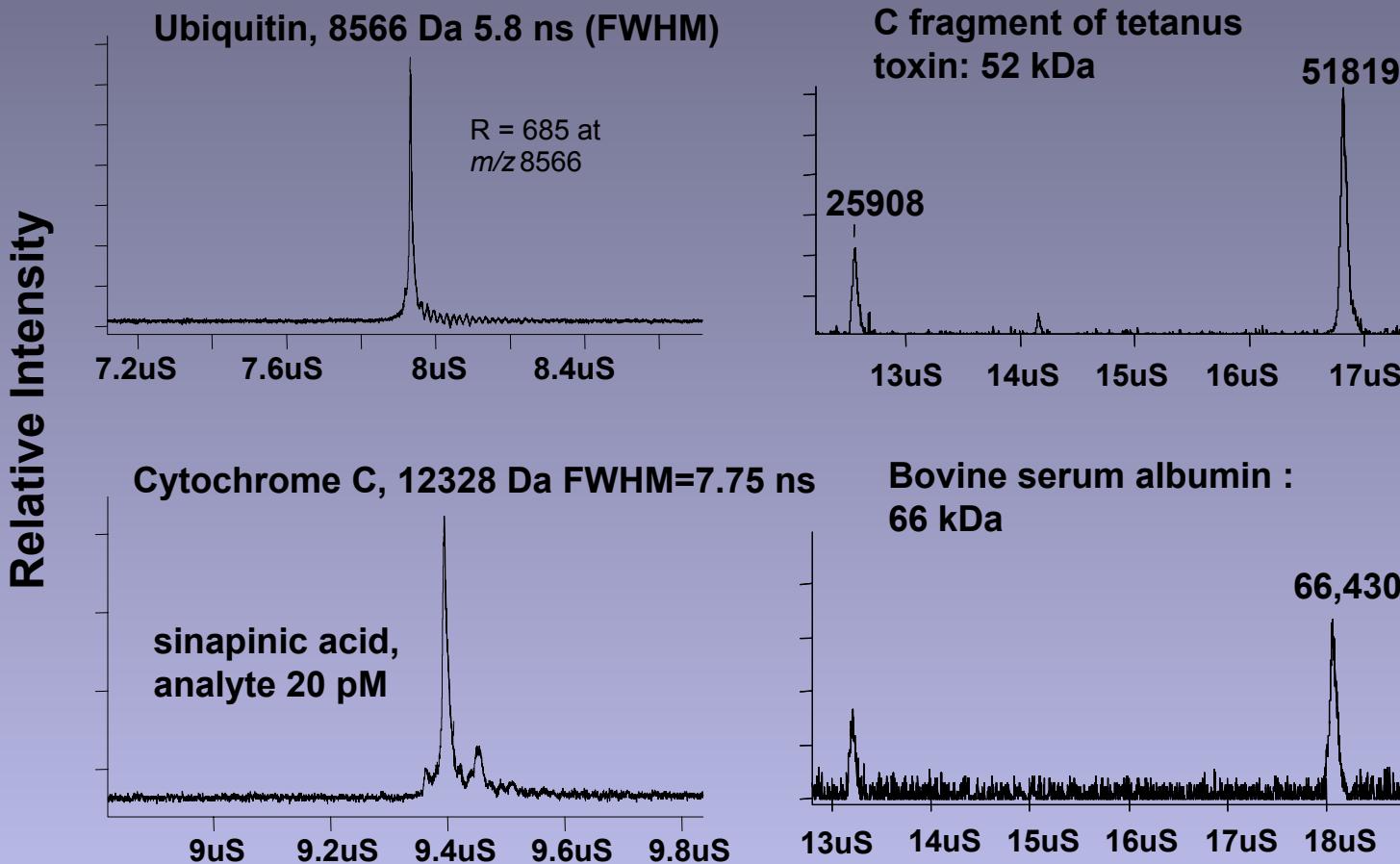


Mass spectrum of ACTH on the miniature linear TOF mass spectrometer shows peak width of only 2 ns!



Pulsed extraction is optimized for mass 4542

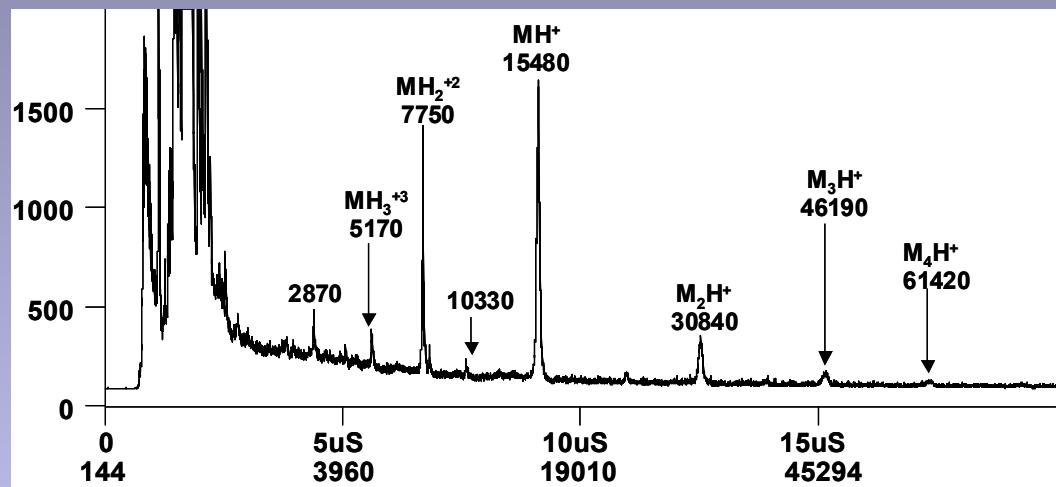
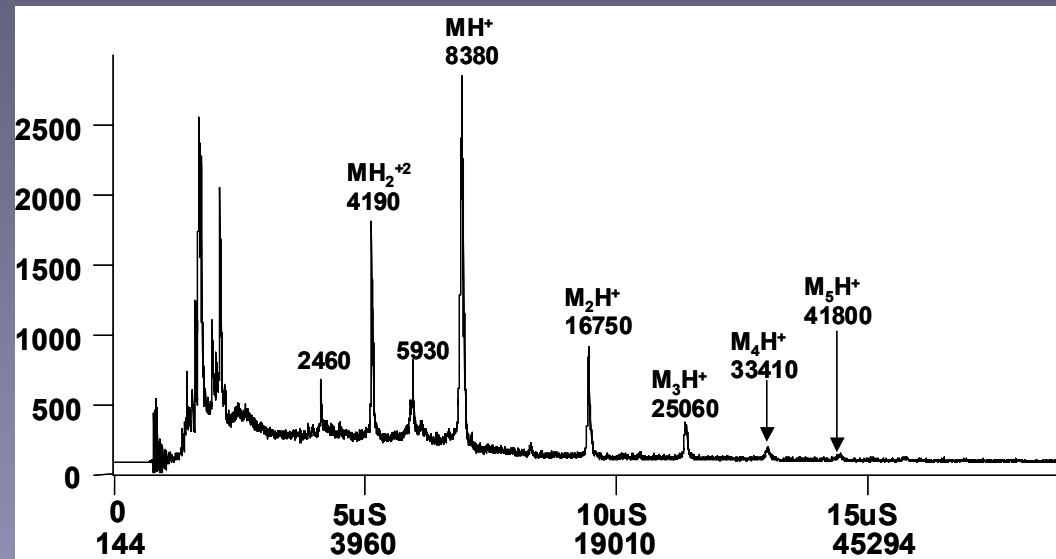
High mass range is retained on a miniature TOF mass spectrometer



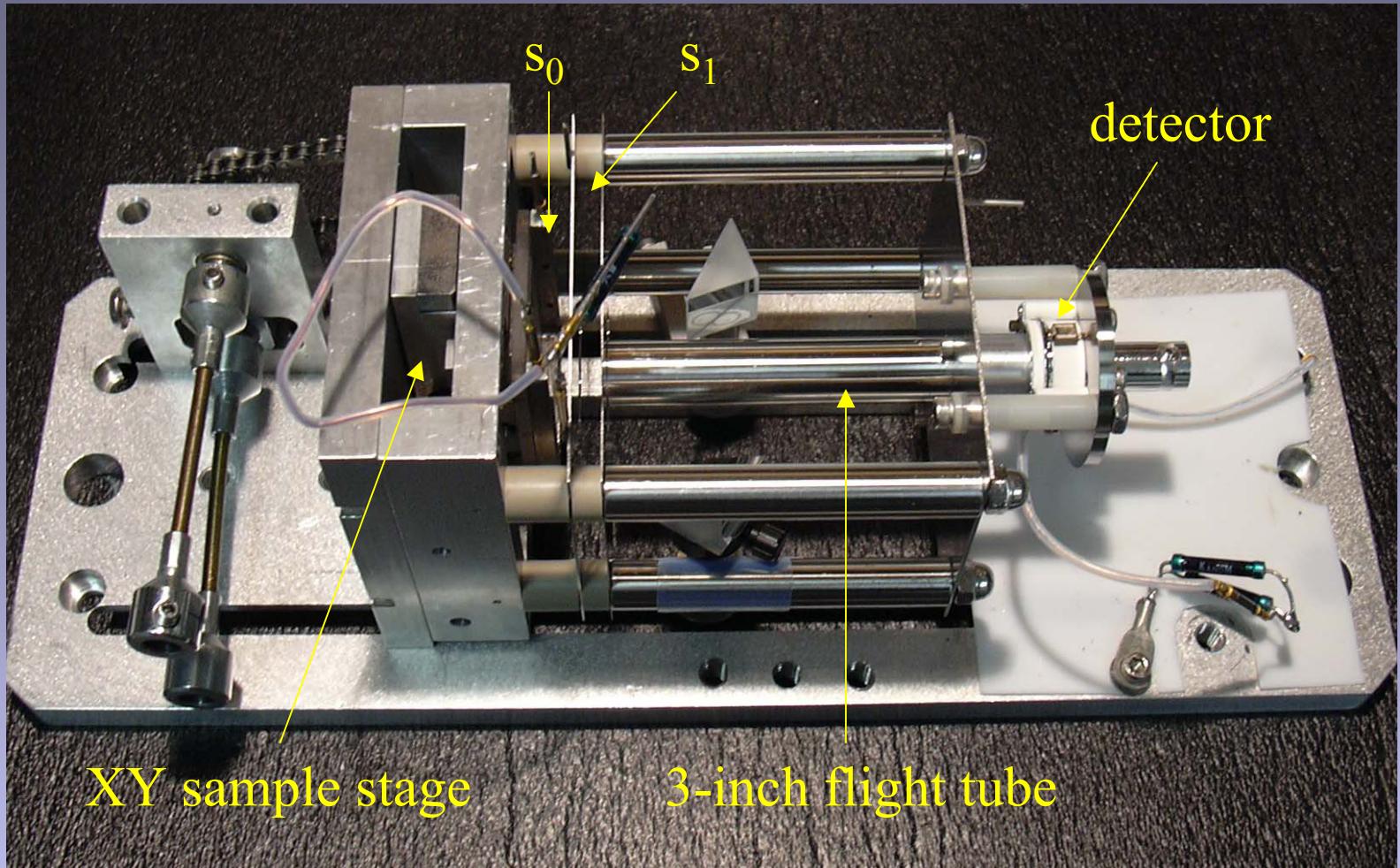
Interleukin-8 and interleukin-2 on the 3-inch miniaturized TOF mass spectrometer

Cytokines (IL-8, IL-2, TNF- α , etc.) and other biomarkers

Clusters and multiply-charged ions span a wide mass range

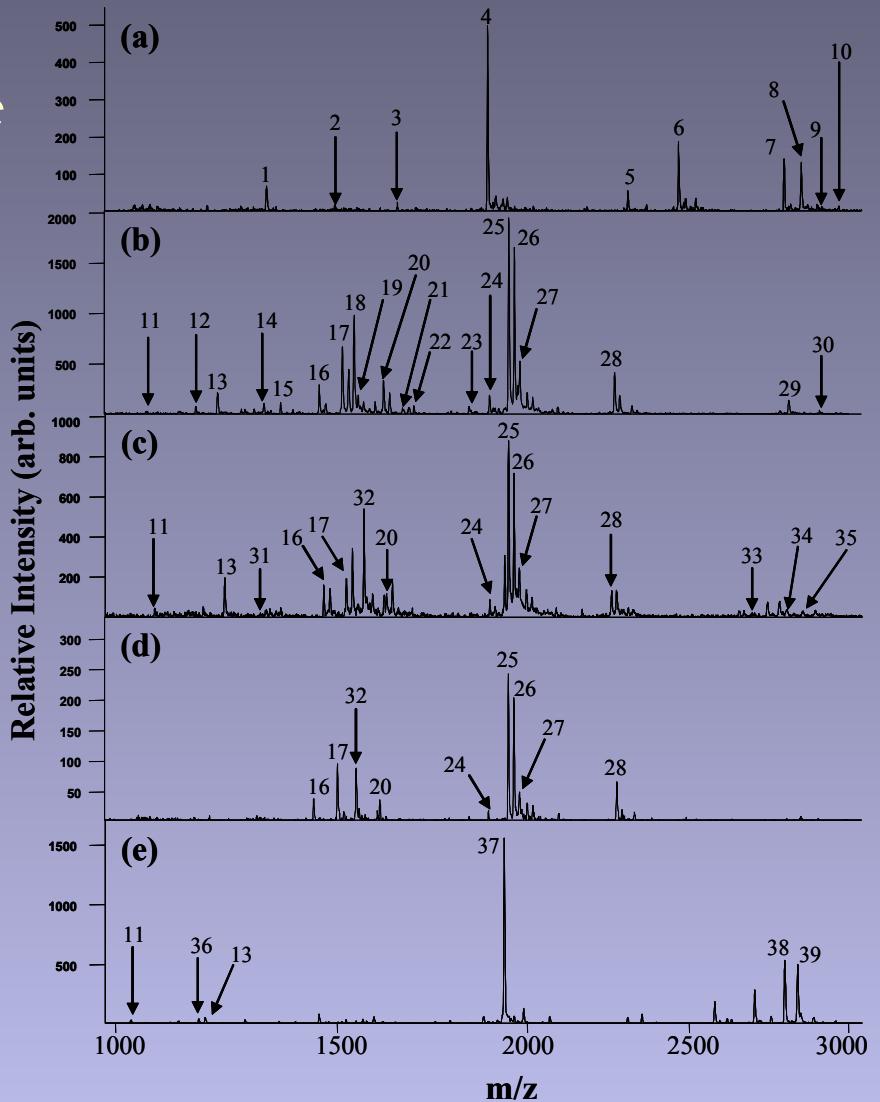


Analysis of Bacillus Spores on the 3-inch TOF



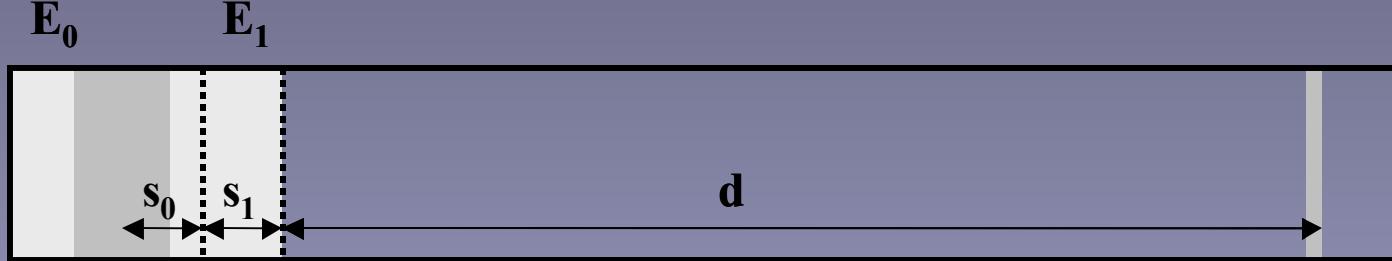
Bacillus Spore *Identification via Proteolytic* *Peptide Mapping with a* *Miniaturized MALDI TOF* *Mass Spectrometer*

Figure 2. MALDI spectra of the tryptic digests generated *in situ* from (a) *Bacillus subtilis* 168, (b) *Bacillus anthracis* Sterne, (c) *Bacillus cereus* T, (d) *Bacillus thuringiensis* subs. *Kurstaki* HD-1, and (e) *Bacillus globigii* spores, and analyzed with the miniaturized TOF mass spectrometer. Peaks that were matched to peptides in the SASP database are numbered 1-39. Peaks that occur in more than one spectrum carry the same number.

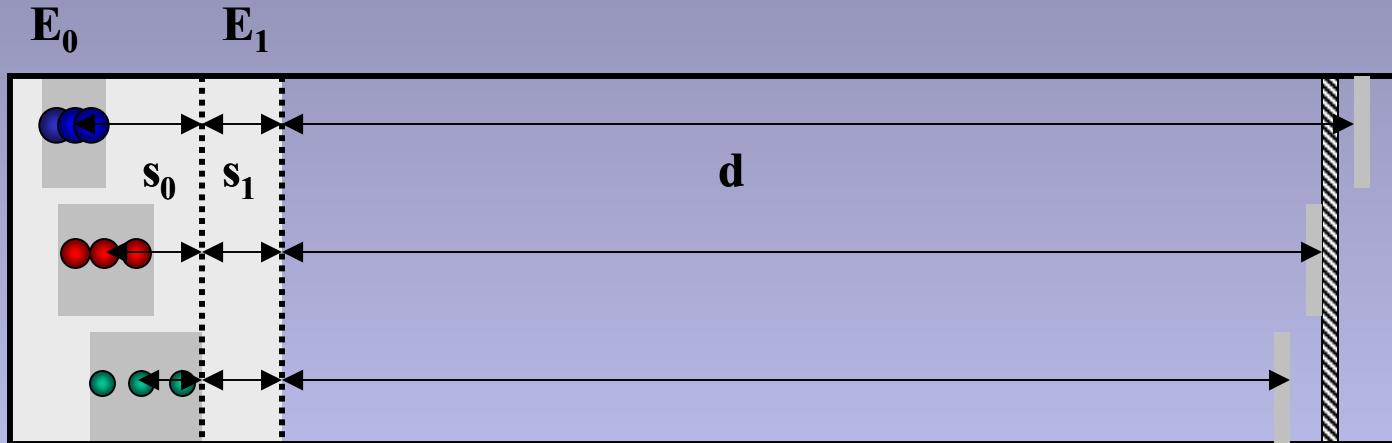


Mass-correlated acceleration (MCA)

Ions distributed in space are focused at a point dependent upon E_0 , s_0 , E_1 and s_1

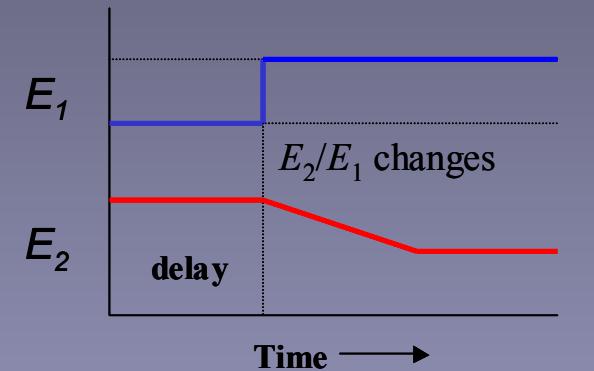


Using ***delayed extraction*** techniques, ions of different mass desorbed from a surface are distributed differently in space:

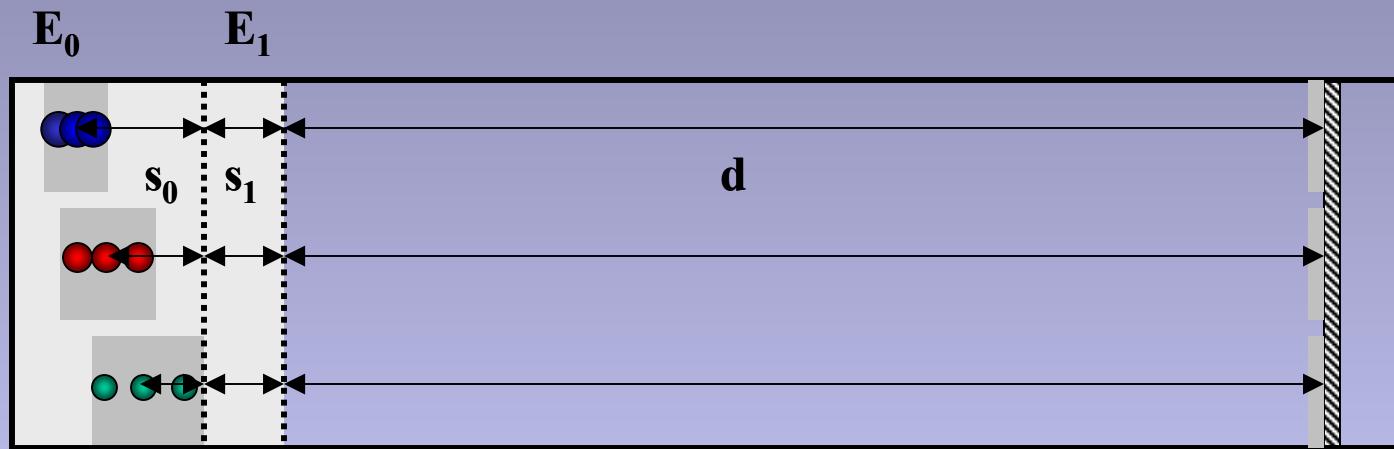


Mass-correlated acceleration (MCA)

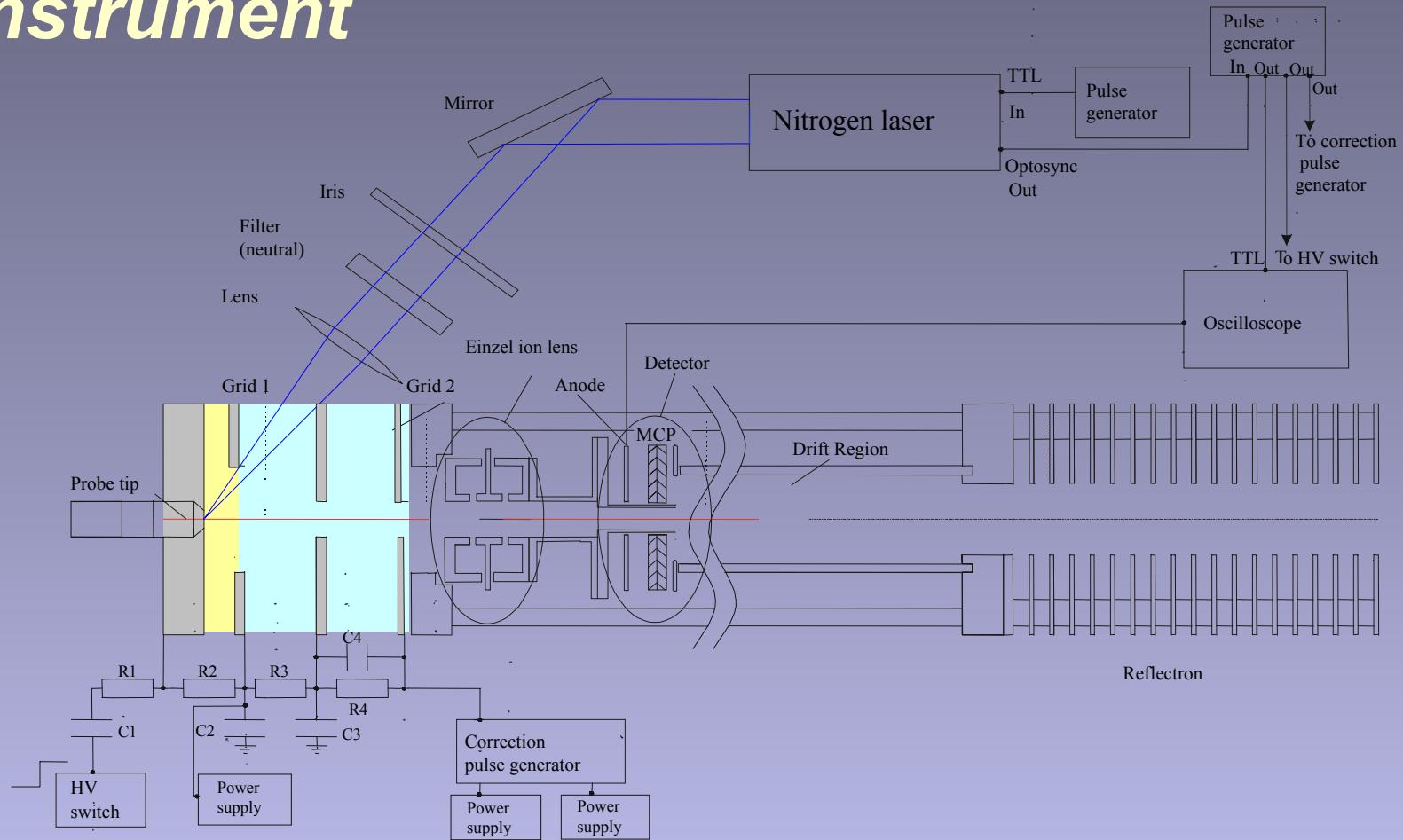
In the **mass-correlated acceleration** (**MCA**) technique, the field in the second ion extraction region or accelerating region changes as each mass enters the region.



Using **mass-correlated acceleration** ions across the mass range are brought into simultaneous focus:



Schematic diagram of the 1 meter MCA instrument



Comparison of pulsed extraction and mass-correlated acceleration on a linear TOF mass spectrometer

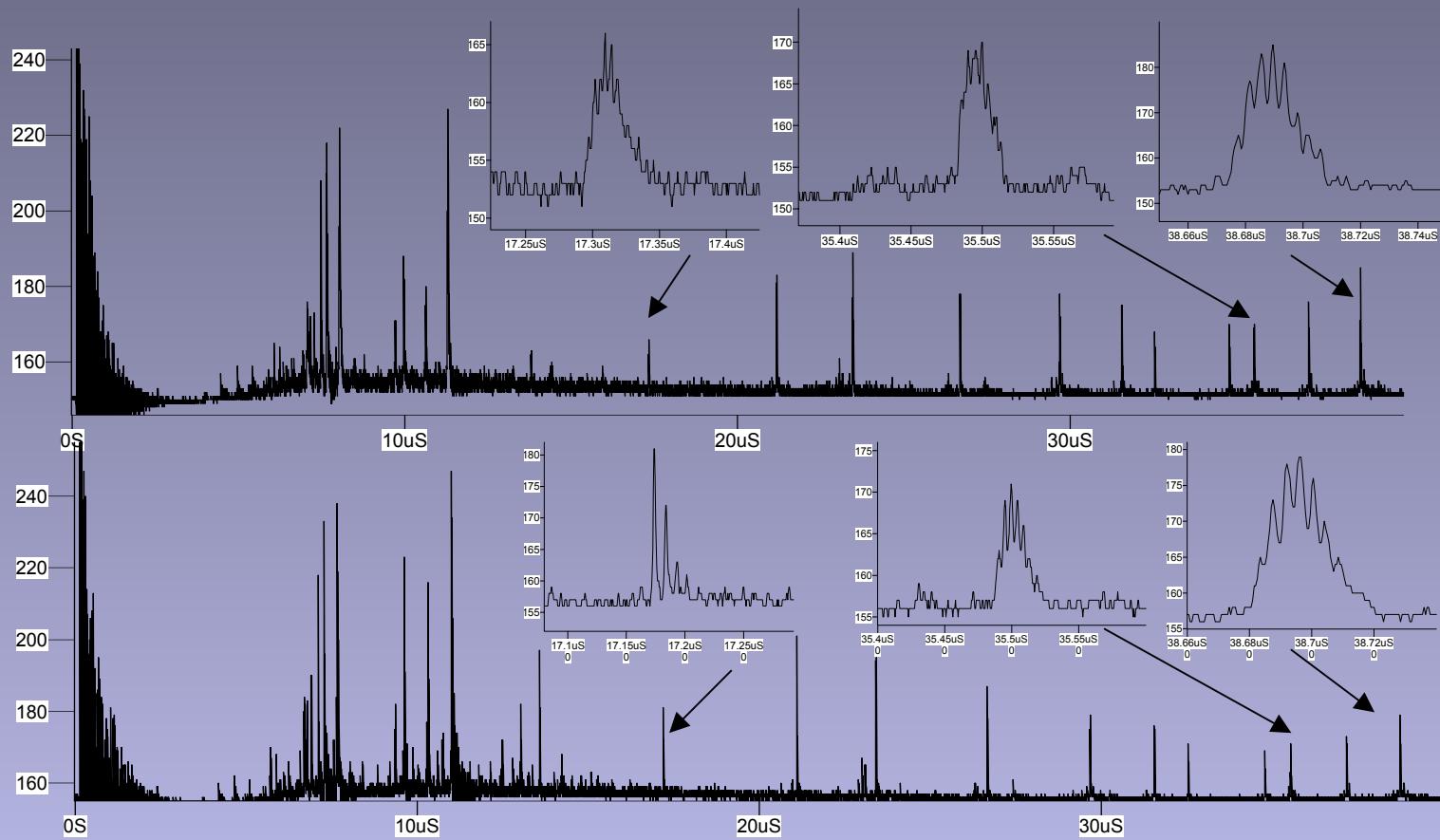


Figure 1. Averaged mass spectra of a mixture of 11 peptides obtained with normal pulsed (delayed) extraction (top) or with *mass-correlated acceleration* (bottom) in a linear TOF. Insets are shown for methionine enkephalin-Arg-Gly-Leu, 900 Da; biocytin-β-endorphin, 3819 Da; ACTH 1-39, 4541Da.

Comparison of pulsed extraction and mass-correlated acceleration on a reflectron TOF mass spectrometer

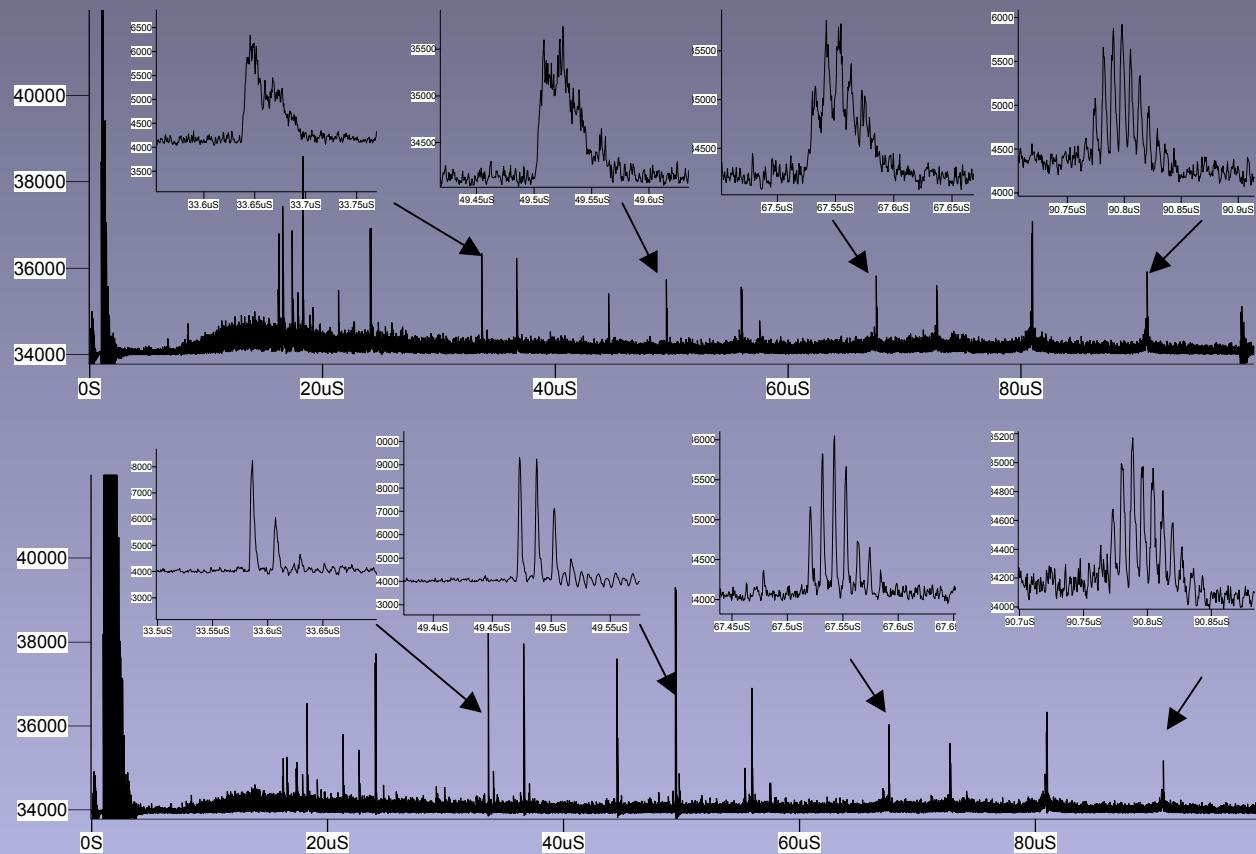


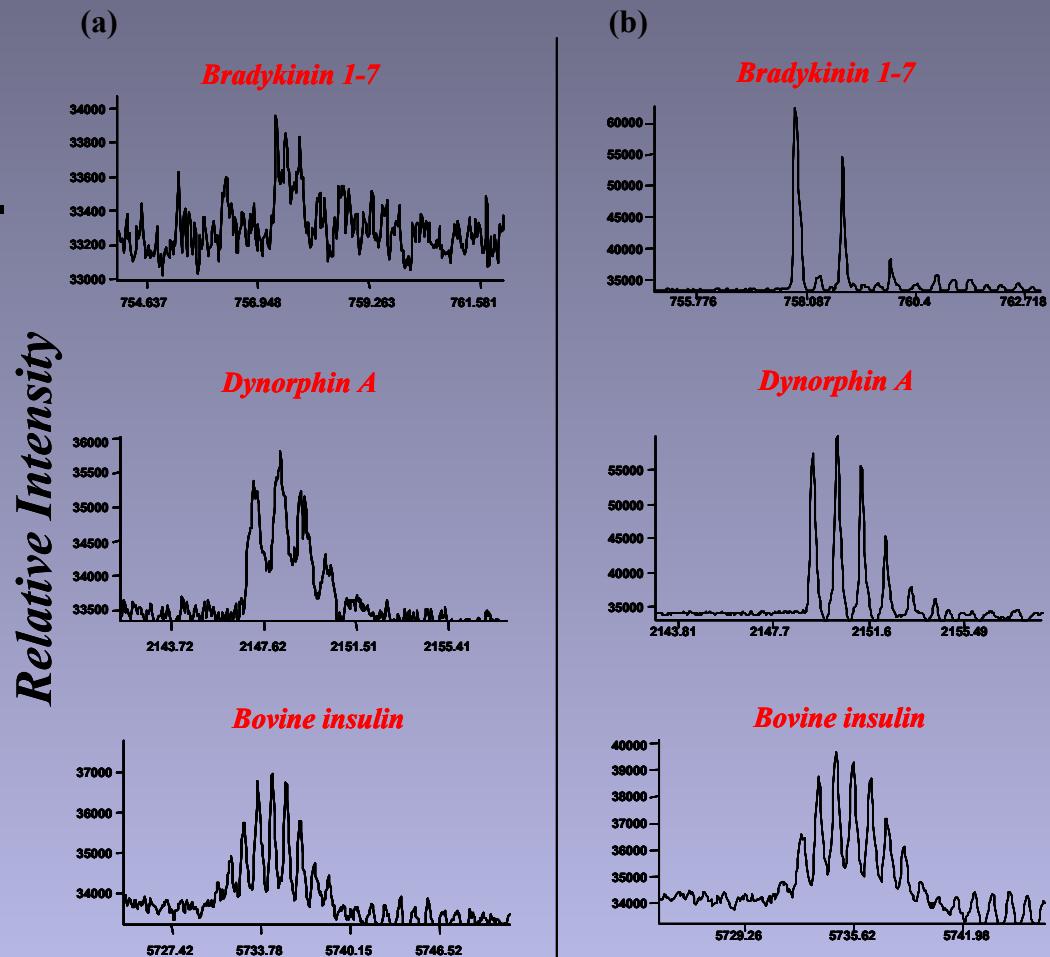
Figure 2. Averaged mass spectra of a mix of 9 peptides obtained with normal pulsed (delayed) extraction (top) or with *mass-correlated acceleration* (bottom) in a reflectron TOF. Insets are shown for bradykinin, fragment 1-7, 758 Da; neurotensin, 1674 Da; somatostatin 28, 3150 Da; insulin, 5734 Da.

Mass-correlated acceleration (MCA)

On a 1 meter TOF instrument MCA provides isotopically-resolved peaks across the mass range:

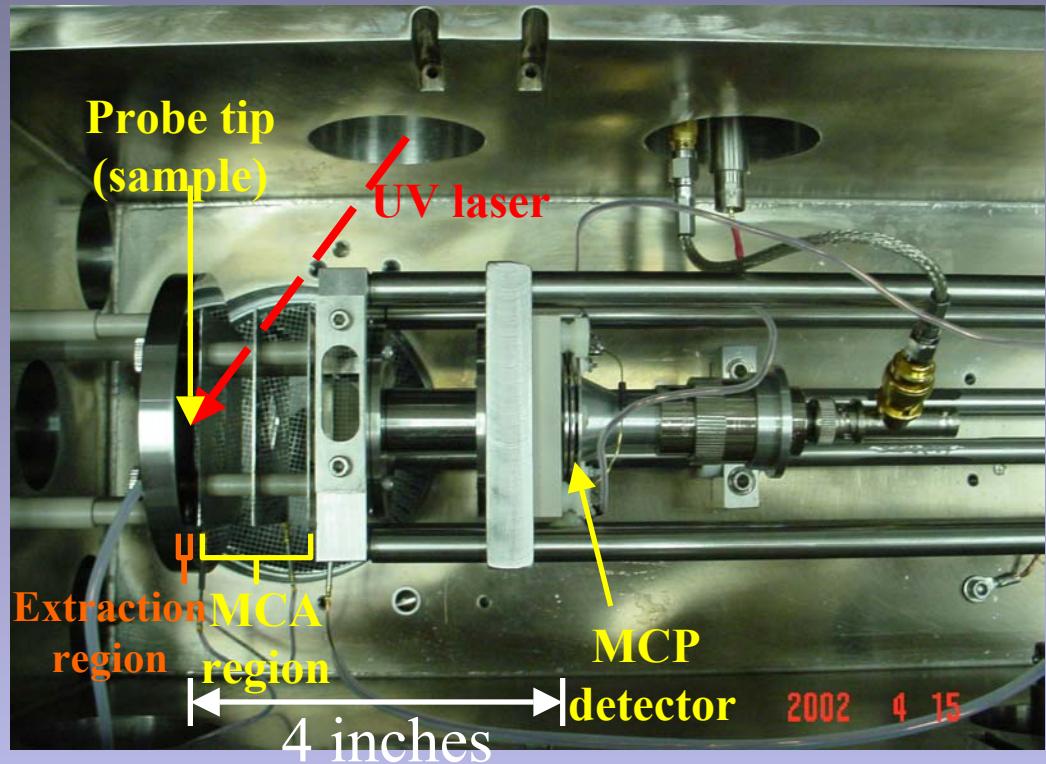
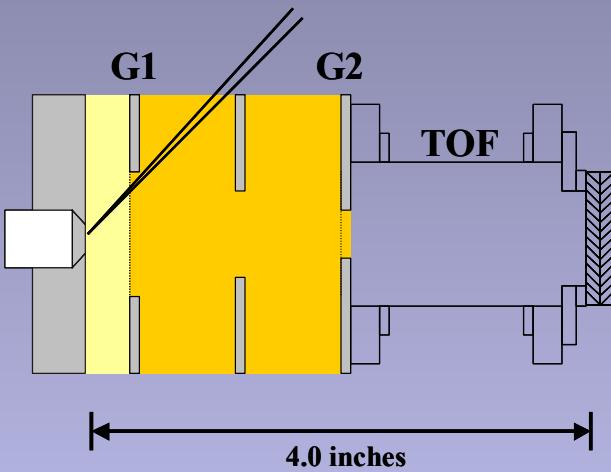
(a) Normal pulsed extraction

(b) Mass-correlated acceleration



Mass-correlated acceleration (MCA) on a miniaturized instrument

**On a 4-inch TOF
instrument: the ion
source is the same
size**

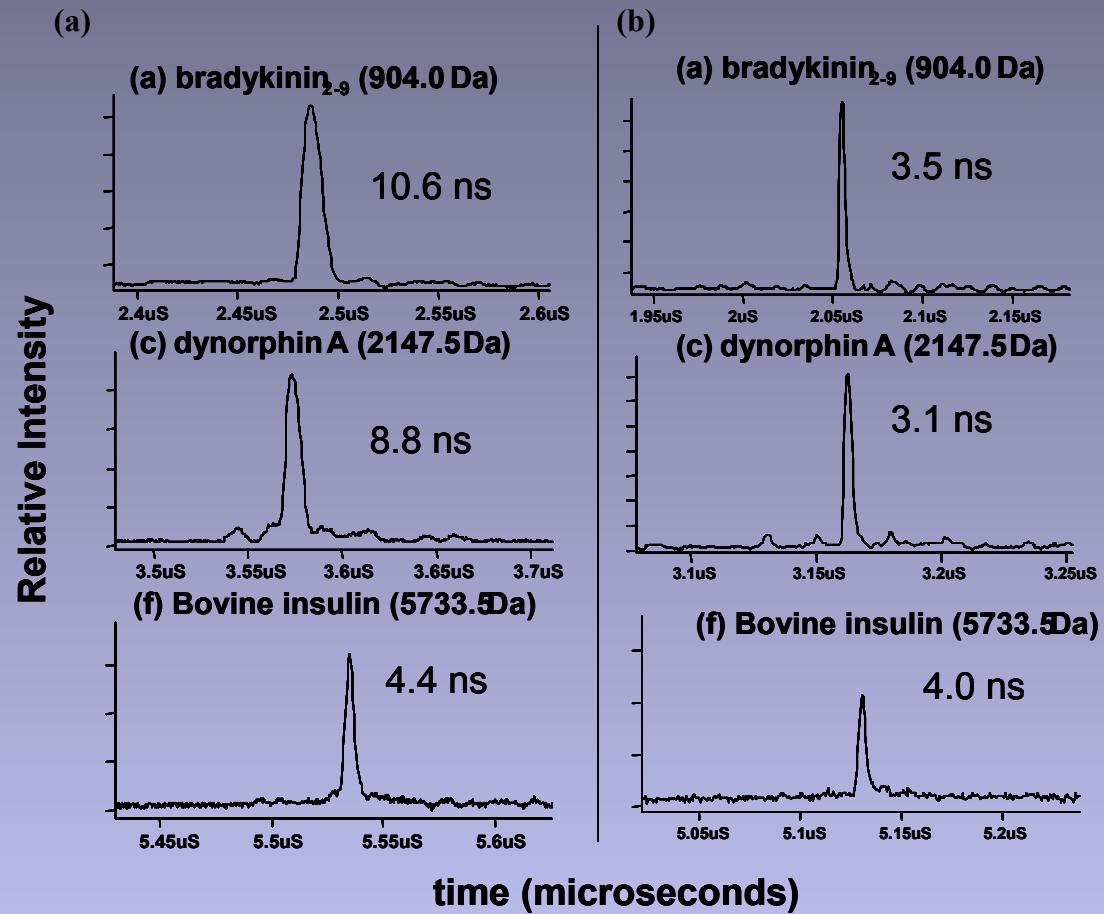


Mass-correlated acceleration (MCA) on a miniaturized instrument

On a 4-inch TOF instrument: unit mass resolution is not observed, but mass resolution is maintained across the mass range

(a) Normal pulsed extraction

(b) Mass-correlated acceleration



Lysozyme (14,313.14) tryptic digest

<i>Peptide Sequence</i>	<i>PE</i>	<i>MCA*</i>
WWCNDGR (937.020 Da)	15.6 ns	3.7 ns
GTDVQAWIR (1046.170 Da)	14.7 ns	3.6 ns
FESNFNTQATNR (1429.488 Da)	12.1 ns	3.4 ns
IVSDGNGMNAWVAWR (1676.890 Da)	11.4 ns	3.7 ns
NTDGSTDYGILQINSR (1754.850 Da)	11.7 ns	2.7 ns
KIVSDGNGMNAWVAWR (1805.064 Da)	11.7 ns	3.5 ns
FESNFNTQATNRNTDGSTDYGILQINSRR (3165.316 Da)	6.8 ns	3.4 ns

*Parameters set
to focus on
 $M+H^+ = 5734.5$*

BSA (66,432.96 Da) tryptic digest: comparison of peak widths using pulsed extraction and mass correlated acceleration.

<i>Peptide Sequence</i>	<i>PE</i>	<i>MCA*</i>
YLYEIAR (928.075 Da)	11.4 ns	3.2 ns
LVNELTEFAK (1164.343 Da)	11.0 ns	4.2 ns
HPEYAVSVLLR (1284.500 Da)	11.6 ns	3.1 ns
HLVDEPQNLIK (1306.504 Da)	8.0 ns	2.8 ns
RHPEYAVSVLLR (1440.688 Da)	9.2 ns	3.1 ns
LGEYGFQNALIVR(1480.706 Da)	10.6 ns	3.2 ns
DAFLGSFLYEYSR (1568.725 Da)	10.8 ns	2.6 ns
KVPQVSTPTLVEVSR (1640.920 Da)	9.4 ns	3.1 ns
MPCTEDYLSLILNR (1668.963 Da)	10.4 ns	3.4 ns
RPCFSALTPDETYVPK (1825.089 Da)	10.3 ns	3.0 ns
DDSPDLPKLKPDPNLTLCDEFKADEK (2832.108 Da)	8.6 ns	4.5 ns

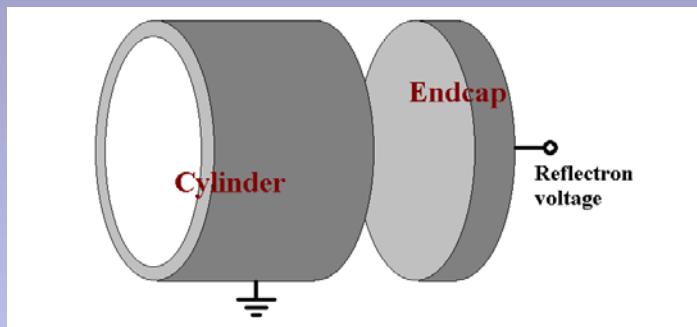
*Parameters set
to focus on
 $M+H^+ = 5734.5$*

Non-linear geometries

Single-stage reflectron: first order focusing of energy eV

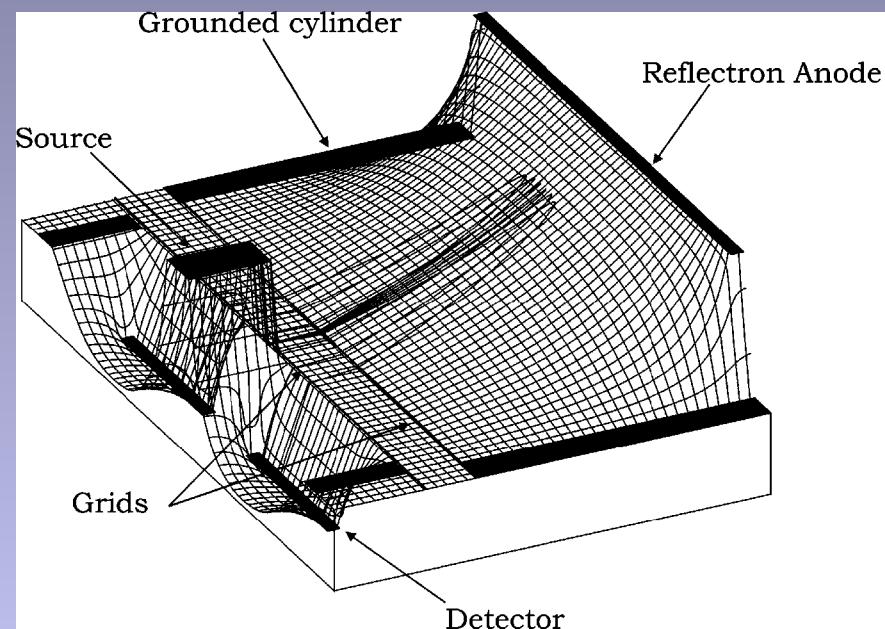
Quadratic reflectron: t is independent of kinetic energy

Endcap reflectron: is nearly quadratic



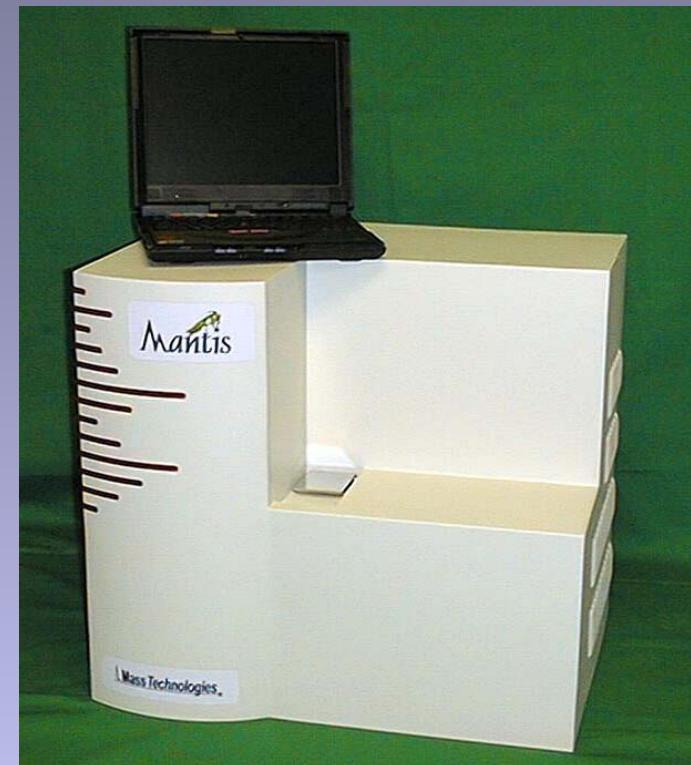
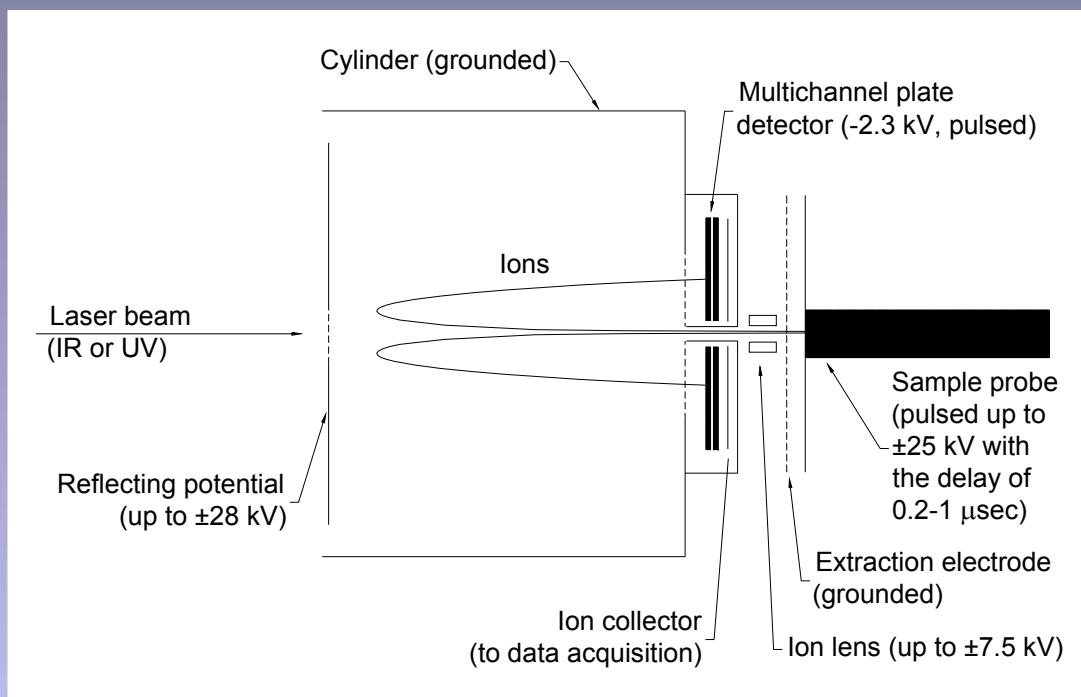
$$t = \left(\frac{m}{2eV} \right)^{1/2} [L_1 + L_2 + 4d]$$

$$t = \pi \left(\frac{m}{2ea} \right)^{1/2}$$



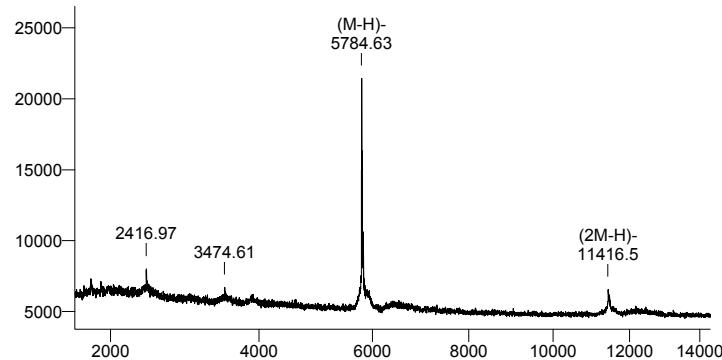
Endcap reflectron TOF

4.5" diameter/3.0" depth
Mass Technologies, Inc.

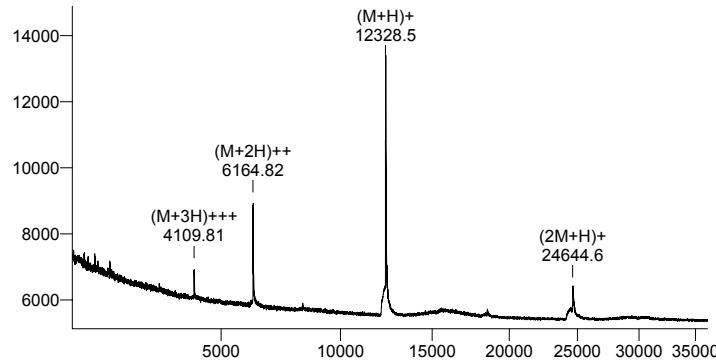


Mass spectra from the endcap reflectron TOF

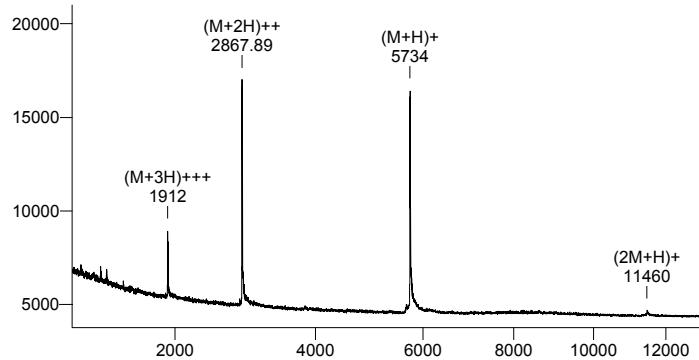
NI UV MALDI mass spectrum of bovine insulin (MW=5,733 Da)



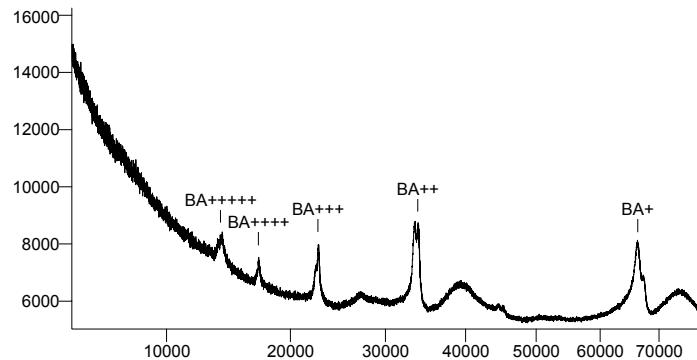
NI IR MALDI mass spectrum of cytochrome C (MW=12,327 Da)



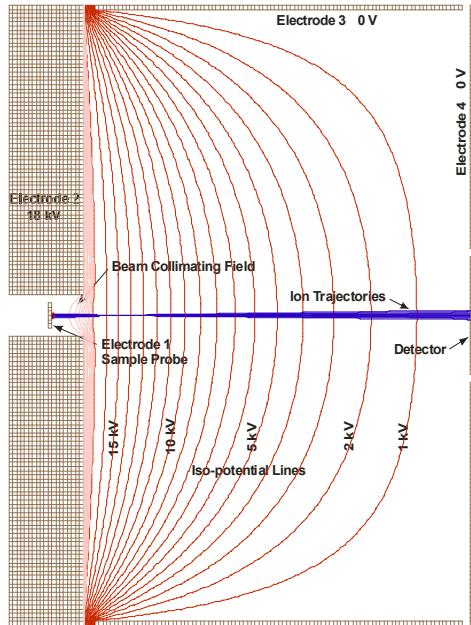
NI IR MALDI mass spectrum of bovine insulin (MW=5,733 Da)



UV MALDI mass spectrum of bovine serum albumin (MW=66,429 Da)



Turning the endcap around: a TOF with a single non-linear field region



JHU technology DM4077: *Non-Linear Time-of-Flight Mass Spectrometer*

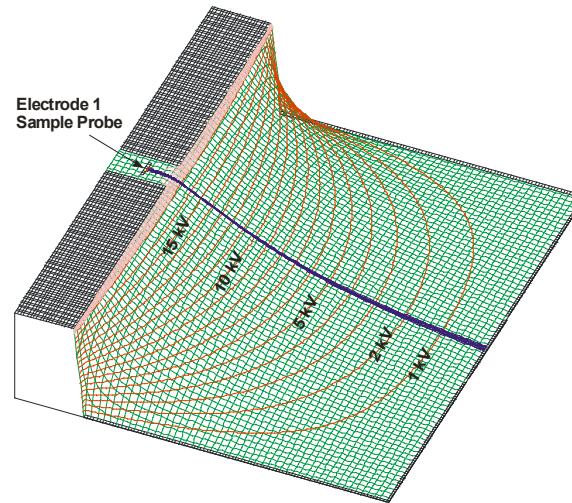


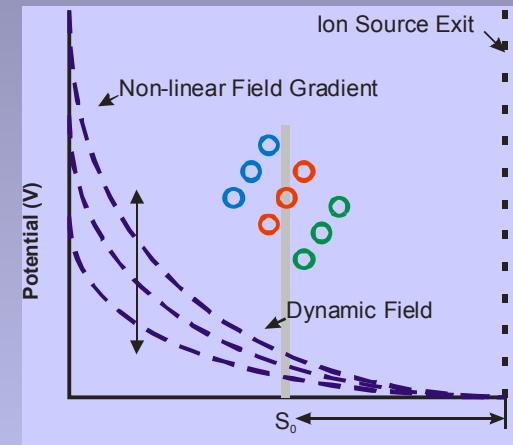
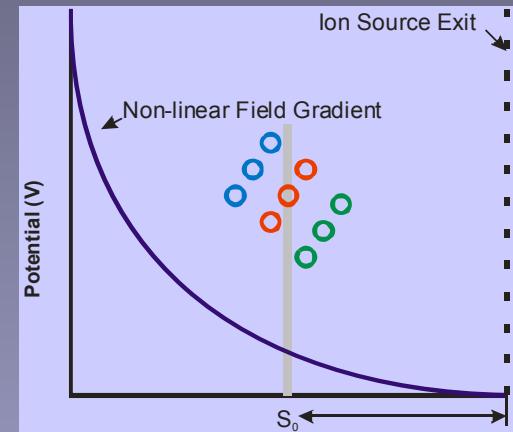
Figure 4: Cross-sectional and 3-dimensional topographical views of the physical geometry and non-linear electrical field distribution for the first embodiment.

An extraction field that is both non-linear in space and time

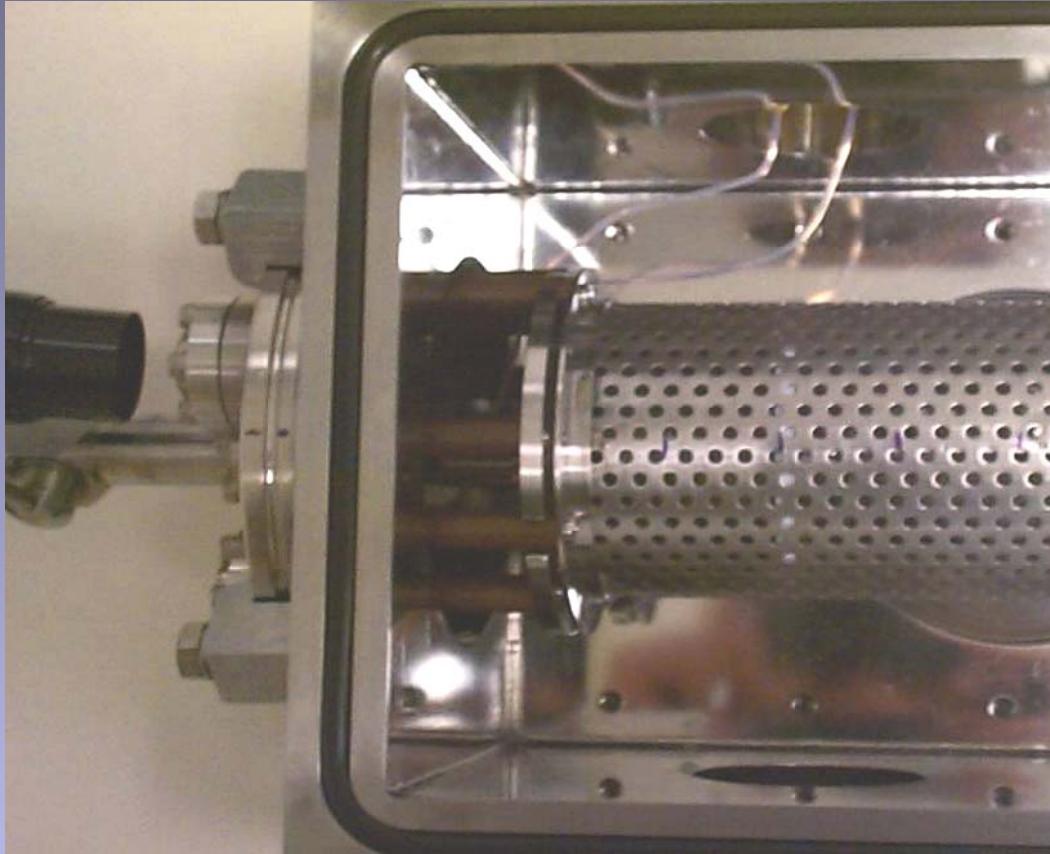
A quadratic field would provide infinite order space focusing at the end of the source region (i.e. there would be no drift region).

When space and velocity are correlated as in MALDI, delayed extraction will provide energy focusing, but still be mass dependent.

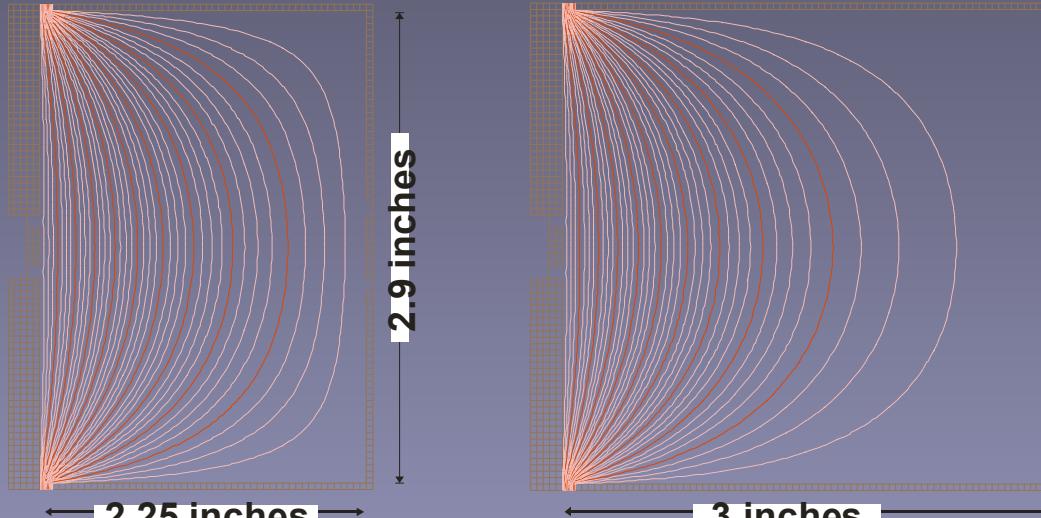
Therefore, need a field that is non-linear in time as well as in space.



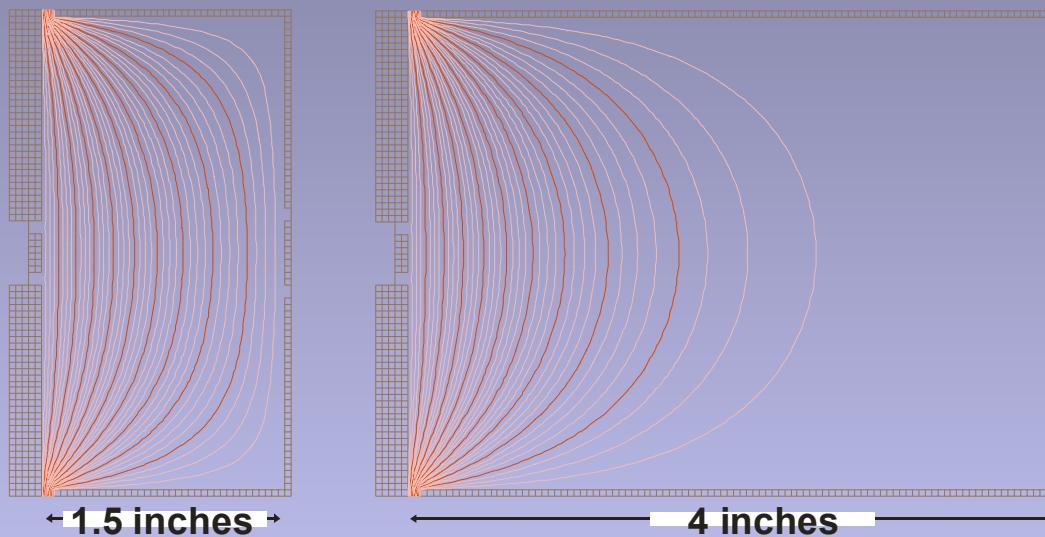
The platform for the “non-linear TOF” has an adjustable aspect ratio



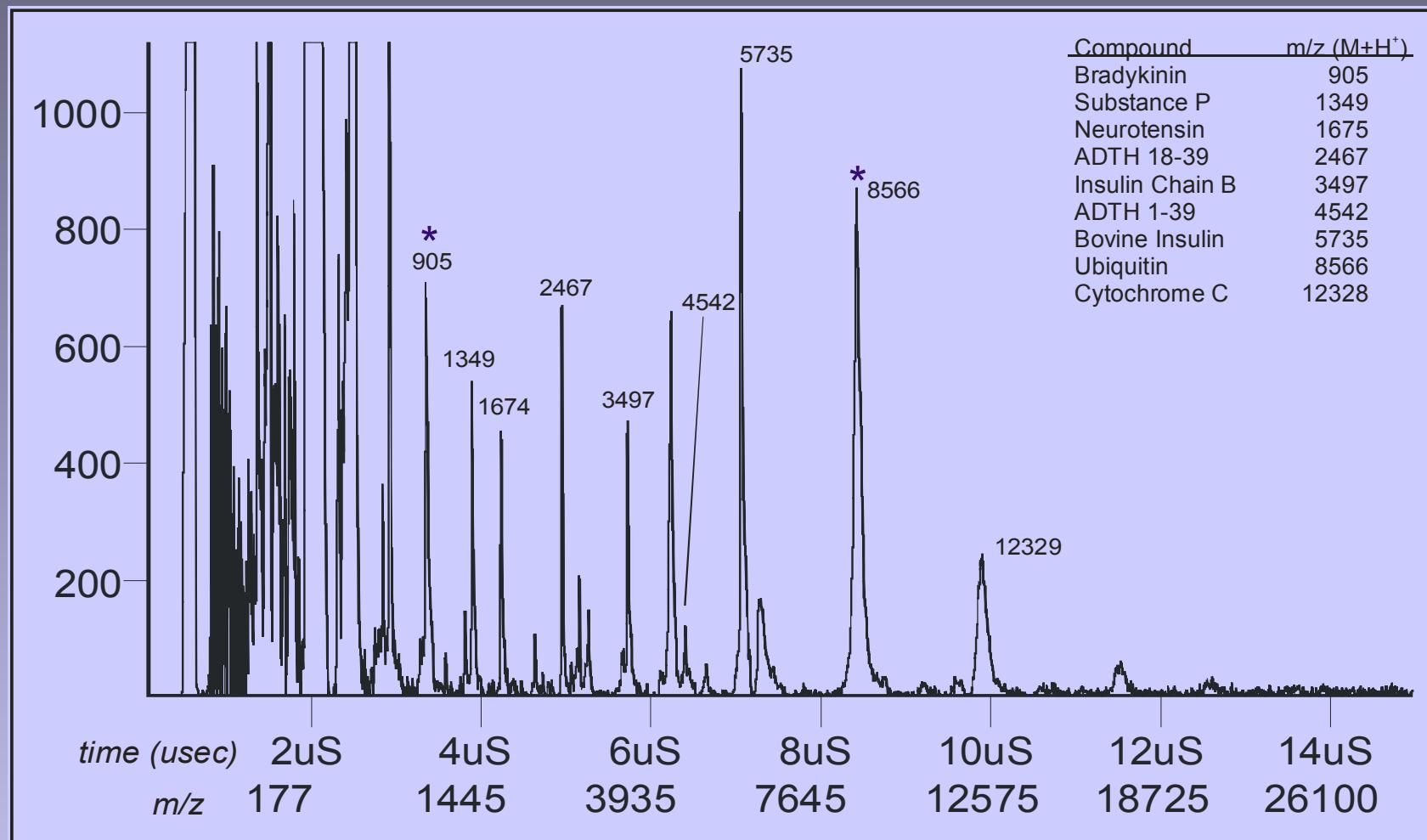
***Infinite order
space focusing
at the end of
the single
region requires
a quadratic
field***



***The optimal
aspect ratio
approximates
this field***

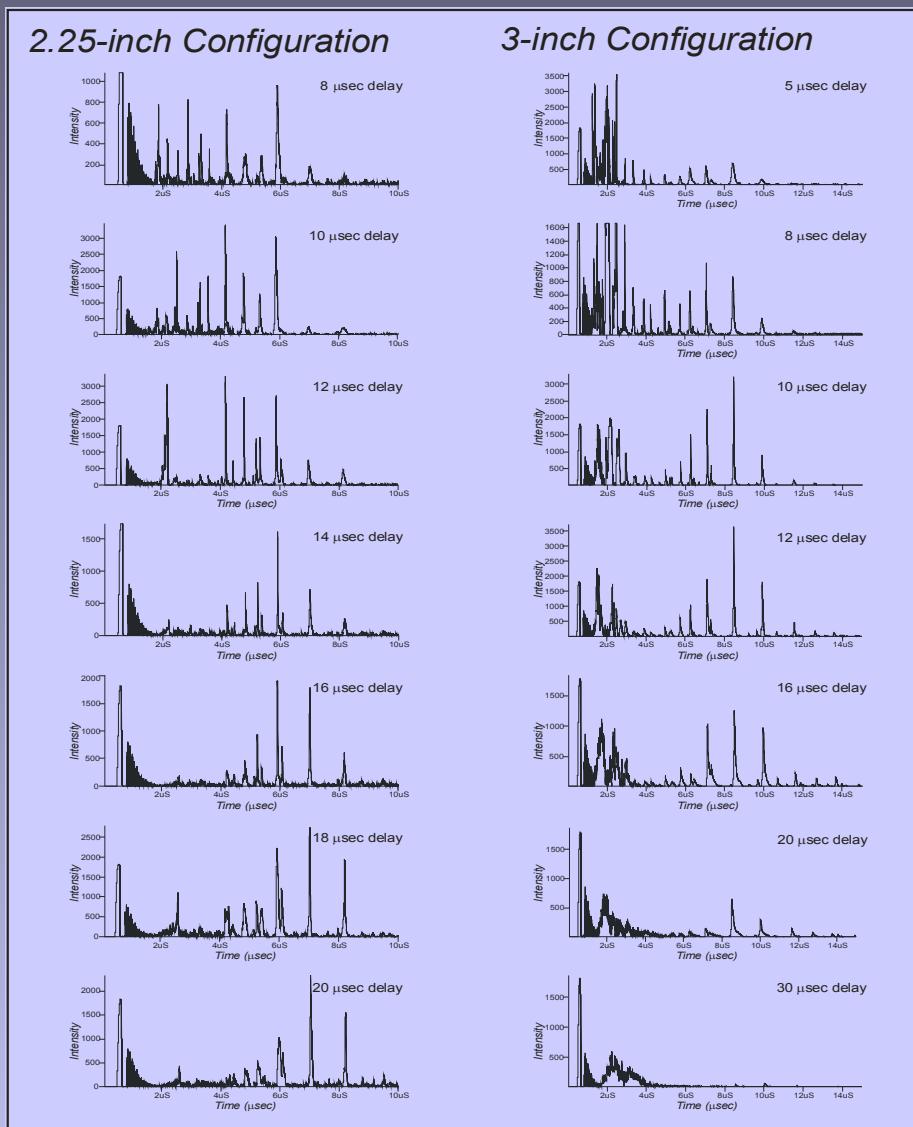


Results for 2.25" with delayed extraction

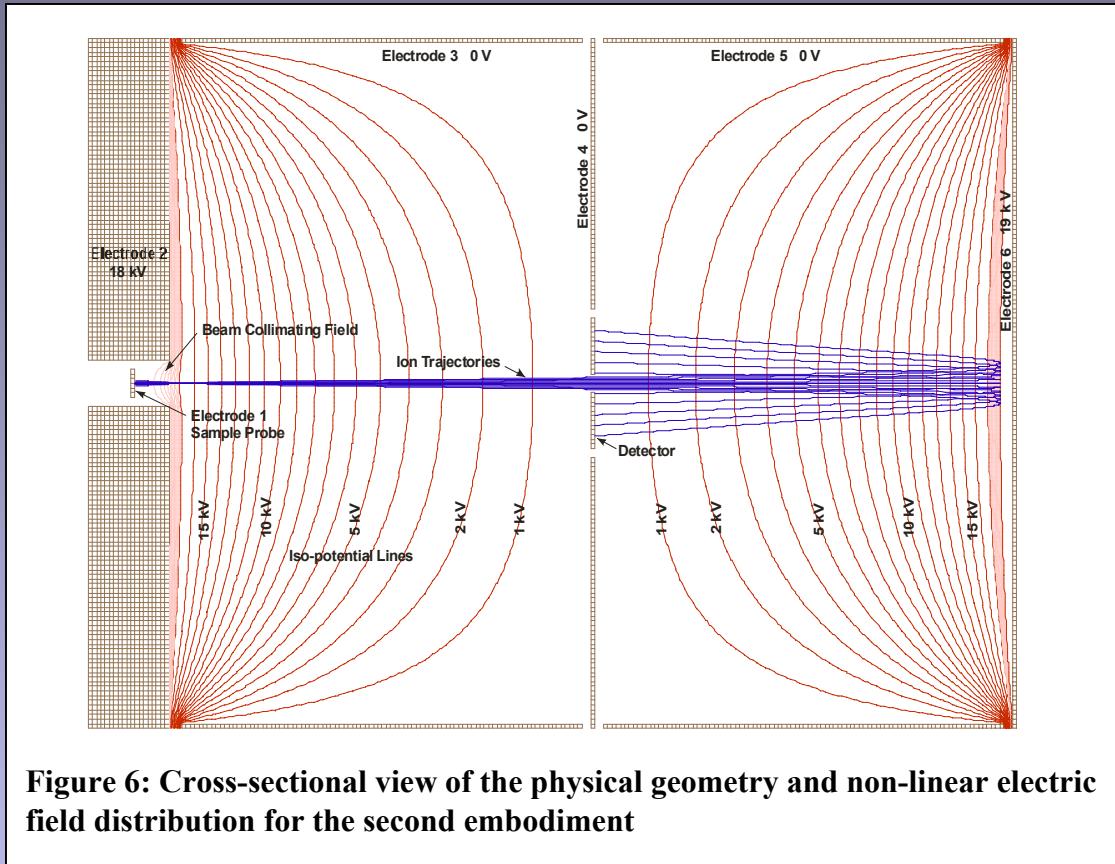


Energy focusing by delayed extraction still shows mass dependence

*Next stage will be
to develop time
dependence of the
non-linear field*



Such an instrument can also be configured as a reflectron or tandem (TOF/TOF) MS



JHU technology
DM4077: *Non-Linear Time-of-Flight Mass Spectrometer*

Acknowledgements:

3-inch instrument

Slava Kovtoun, Mari Prieto, Robert English

Mass correlated instrument

Slava Kovtoun, Robert English

Endcap reflectron mass spectrometer

Timothy Cornish, Vladimir Doroshenko (MassTech)

Non-linear instrument

Ben Gardner

The MAMS Laboratory



Mass Spectrometry of Synthetic Polymer Mixtures
NIST Polymer Workshop, November 13, 2003



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